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(71) Applicant: **N.V. Nutricia**
2700 MA Zoetermeer (NL)

(72) Inventors:
- **Van Hoey-De-Boer, Klaske Anna**
  3443 BD Woerden (NL)
- **Hageman, Robert Johan Joseph**
  2742 EV Waddinxveen (NL)

(74) Representative:
de Bruijn, Leendert C. et al
Nederlandsch Octrooibureau
P.O. Box 29720
2502 LS Den Haag (NL)

(54) Probiotic nutritional preparation

(57) The invention relates to a nutritional preparation with health promoting action, in particular with respect to the prevention and treatment of disorders of the gastrointestinal tract, comprising 10^6-10^{14}, preferably 10^7-10^{13} viable cells, per gram of the total preparation, of of each of the following micro-organisms:

- Bifidobacterium;
- Enterococcus faecium; and
- a Lactobacillus strain that produces predominantly dextro-rotary lactate.

The nutritional preparation can further comprise a corresponding amount of cells of a Lactococcus strain or a Micrococcus strain.

Also, the preparation preferably contains prebiotic compounds, as well as substances that inhibit bacterial adhesion to the wall of the gastrointestinal tract.

The preparation can be in the form of a food supplement, a ready-to-use food composition, an infant formula or a tube feeding.

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[0016] The Lactobacillus strain used is preferably such that it predominantly, more preferably exclusively produces dextro-rotary (L+) lactate. By this is meant that of the lactate produced, less than 5%, preferably less than 2% is levo-rotary lactate. Of course, the micro-organism can produce other metabolites besides the L(+) lactate.

[0017] The use of these Lactobacillus strain is preferred because in certain disorders of the GI-tract, such as short bowel syndrome, carbohydrate malabsorption or in cases of carbohydrate overload, levo-rotary lactate will not be disgested (sufficiently), which can cause adverse reactions such as acidosis.

[0018] Examples are Lactobacillus rhamnosus (previously called Lactobacillus casei sbsp rhamnosus), such as the preferred strains ATCC 7469 and 271 DSM 6549; Lactobacillus [GG]; L. casei NCIMB 8823 and L. casei DSM 20244.

[0019] Other suitable strains of the above micro-organisms are mentioned in the prior art cited above, which is incorporated herein by reference.

[0020] As further discussed below, these micro-organisms, as well as any other micro-organisms used, preferably have good adhesion properties to the gastrointestinal wall, in particular mucous membranes thereof and/or any receptors thereon. Also, the bacteria used will preferably by themselves have no adverse effect on the health of the person or animal to be treated. As mentioned, this can be achieved by using micro-organisms which are endogenous to the gastro-intestinal tract (or strains derived therefrom) or micro-organisms which are present in food products, such as lactic acid bacteria.

[0021] These micro-organisms can be obtained in any manner known per se, such as cultivating them in suitable media, preferably in such a way and using such media that the microbial preparations thus obtained are suitable for adminstration to humans and/or animals, and especially are able to colonize the desired parts of the gastrointestinal tract.

[0022] The bacteria are preferably used in amounts (relating to the total number of bacteria administered) of 0,1-20 parts Bifidobacterium to 0,1-20 parts Enterococcus faecium to 0,1-20 parts Lactobacillus, more preferably in about equal amounts.

[0023] These bacteria are preferably each administered in an amount of 10^7 -10^{13} cells/day, preferably 10^{10} - 10^{11} cells/day, in 1 to up to 10 doses/day, both for adults as well as children. The nutritional composition of the invention, in particular the amounts of micro-organisms therein per unit of volume or weight, is preferably such that such a daily dosis can easily be provided, as well as administered.

[0024] Besides the three types of micro-organisms mentioned above, the preparations of the invention can contain one or more further micro-organisms which prevent the growth and/or the adherence of pathogenic microoganisms in the gastrointestinal tract. Specific examples are non-pathogenic E.coli strains, in particular E.coli strains that do not produce any toxins and do not contain any plasmids.

[0025] Also very suitable are Lactobacillus strains (including those that do not predominantly produce dextro-rotary lactate), such as Lactobacillus GG, of which strain ATCC 53103 is preferred, as well as other lactic acid bacteria, for instance Lactococcus species/strains, such as Lactococcus lactis, of which strain LW-P53 is preferred.

[0026] Instead of, or in combination with, Lactococcus strains, Micrococcus strains can also be used, such as M. luteus (of which M. Luteus ATCC 4698 is particularly preferred), M. varians, M. kristinae or M. sedentarius.

[0027] Other suitable strains of micro-organisms are mentioned in the prior art cited above, which is incorporated herein by reference.

[0028] These further micro-organisms can also be present in the preparations of the invention in amounts of 10^7 - 10^{13}, preferably 10^8-10^{12}, more preferably 10^{10} - 10^{11} viable cells/gram of the total preparation, most preferably in amounts in the range of the three micro-organisms mentioned above. They are also administered in amounts/doses as mentioned above.

[0029] The preparation of the invention is preferably in the form of a food supplement, or in the form of a food or food composition which as such is ready for consumption.

[0030] When the preparation of the invention is in the form of a food supplement, it can be in a form for separate administration, such as a capsule, a tablet, a powder or a similar form, containing preferably a unit dose of the micro-organisms, containing 10^7-10^{13} cells/dose, preferably 10^{10} - 10^{11} cells/dose.

[0031] The food supplement can also be in the form of a powder or a similar form, which is added to, or mixed with, a suitable food (composition) or a suitable liquid or solid carrier, for the preparation of a food which is ready for consumption.

[0032] A preferred embodiment is a freeze-dried powder of the micro-organisms, which can be in the form of a sachet, or which can be incorporated in a capsule or a tablet or another dry administration form. These freeze-dried preparations can be obtained using suitable techniques and can contain suitable adjuvants known per se, for instance cryoprotectants such as maltose.

[0033] For instance, the invention in the form of a food supplement can be in the form of a freeze-dried powder, which is reconstituted using a suitable liquid, such as water, oral rehydration solution, milk, fruit juice, or similar drinkable liquids. It can also be in the form of a powder which is mixed with solid foods, or foods with a high water-content, such as fermented milk products, for example yoghurt.

[0034] The nutritional preparations of the invention can also be in the form of a food which is ready for consumption. Such a food can for instance be prepared by

tioned above; a protein; or a seperate precursor compound known per se.

[0051] The preparations of the invention are preferably lactose-free, and do not have a high osmolality (preferably less than 400 mosm/l, more preferably less than 300 mosm/l).

[0052] All micro-organisms used in the invention are preferably resistant to degrading conditions in the gastrointestinal tract, such as amylase, stomach acids, bile (salts), lipases and/or pancreatic fluid, so that they can pass the stomach and have their beneficial influence on the gastrointestinal tract. Therefore, they can be used as such without precaution as to their passing the stomach. However, preferably the oxygen-sensitive Bifidobacteria are encapsulated for passage of the stomach by means of a suited delayed release encapsulation. Suitable compounds for encapsulation are chitosan, maltodextrin, dextrins, lipids, polylactate, and poly- or oligosaccharides.

[0053] According to a specifically preferred embodiment, all micro-organisms used are encapsulated, when used in liquid or moist products. This prevents that the micro-organisms, which are present present in the preparations of the invention as living or at least viable cells, start growing in and/or fermenting the food product, thereby reducing its shelf-life. This is a problem with almost all ready-to-use type preparations of the invention, in particular when a food or food base is used that provides suitable conditions for bacterial growth, such as an aqueous medium and sources of nutrients for the micro-organisms (which will be the case with almost all consumption foods). It can however also be a problem with nutritional supplements for household use, which are added to food products, and then kept for some time, as the bacteria of the invention can appreciably start fermenting and/or degrading any suitable food medium within half an hour. For instance, when the preparations of the invention are combined with food products on the basis of cereals, in particular when these have a high water content, the shelf life is drastically reduced.

[0054] In view of this, encapsulation of the micro-organisms can provide an improved shelf-life of two years or more, in particular for food products which are ready for consumption. They also improve the safety of supplements for adding to food products prior to consumption. Also, such encapsulation can even further improve the resistance against stomach fluids and/or pancreatic fluid.

[0055] Suitable compounds for encapsulation for improving the shelf life, as well as methods for carrying out the encapsulation, are known in the art. Besides the compounds mentioned above, preferred compounds include combinations of chitosan and maltodextrine, and the like.

[0056] Although the invention is not limited to any specific explanation, it is assumed that the probiotic micro-organisms produce their prophylactic and therapeutic effect by competing for nutrients etc., and in general providing surroundings in the gastrointestinal tract which are not suited for the growth of the pathogens, or can even kill them.

[0057] The probiotic micro-organisms also adhere to the walls of the gastrointestinal tract, in particular with the mucous membranes and/or receptors thereon, and thereby compete with the pathogenic micro-organisms under the dynamic conditions present in the GI-tract. This will prevent that the pathogens infect cells, and will also speed up clearance of the pathogens from the gastrointestinal tract. This prevention of adherence (by both living and dead pathogens, as well as fragments thereof) can be increased considerably by including anti-adhesive agents in the compositions. By including specific fibres, the beneficial micro-organisms will have sufficient substrate available for growth and produce factors that can heal the damaged gut tissue.

[0058] The preparations of the invention will in general have a positive influence on the gastrointestinal tract. For this purpose, they can further contain health improving compounds known per se, such as medicaments etc. In particular, the preparations can contain compounds which have a beneficial influence on the gastrointestinal tract, such as glutamine/glutamate or precursors thereof.

[0059] The preparations of the invention can be used for preventing or treating gastrointestinal disorders, such as gastrointestinal infections, diarrhoea, systemic infections, or disturbance in the immune system, in particular those caused by pathogens such as such as enterotoxigenic E.coli strains, rotavirusses, Clostridia, Salmonella or Campylobacter species.

[0060] Also, an insufficient level of probiotic micro-organisms in the gastrointestinal tract can lead to an increased permeability of the gastrointestinal wall, which can increase the immunogenic and allergenic properties of food components or other orally administered compounds. The preparations of the invention can also be used to treat or prevent these phenomena.

[0061] Also, as is known, treatment with antibiotics, in particular oral antibiotics, can disturb or destroy the gastrointestinal flora. The preparations of the invention can either be used to prevent this (usually by administration in combination with the antibiotics); for preventing the colonisation by harmful organisms after treatment is ended; or for restoring the gastrointestinal flora after antibiotic treatment.

[0062] The main advantage of the preparation of the invention is that the different micro-organisms used colonise and grow in different parts of the gastrointestinal tract, and therefore provide overall treatment and/or protection of the gastrointestinal tract. For instance, Lactobacillus, Lactococcus or Micrococcus species only grow in the presence of oxygen, whereas Bifidobacteria and enterococci prefer anaerobic conditions. Lactobacilli, lactococci and micrococci can already settle in the mouth, and preferably settle in the small intestine, in

40 kg magnesium chloride
3 kg zinc citrate

- 10 kg spraydried cranberry extract
- 170 kg Novelose and 40 kg Soy polysaccharide.

[0072] This mixture is packed in sachets of about 2.9 g which are used together with the sachets from example 4.

[0073] Two hundred ninety (290) kg of the mixture can also be mixed with 750 kg of the mixture of example 4 which is packed in sachets of about 10.5 g, which can be used by persons as described in example 4.

Example 6: Fermented complete food

[0074] By methods that are known in the art a complete liquid formule is composed based on caseinats, vegetable oils, maltodextrin and vitamin/mineral premixes.

[0075] Two thousand (2000) litres of this liquid composition is fermented during 4 hours at 35-42°C by inoculating with a sufficient amount of the cultures as described in example 1. The fermented product is pasteurized and packed aseptically in cartons.

Example 7: Infant formula with probiotics

[0076] To 1000 kg of a spraydried infant formula is dry blended 10 kg of the mixture as obtained in example 2 and packed in tins or cartons as known in the art.

Example 8: Tube feeding with probiotics

[0077] The microorganisms as described in step 2 of example 1 are encapsulated by methods known in the art to become sustained released, for example by including them in small particles coated with a fibre type constituent or chitin, which dissolves slowly in the stomach but mostly in the lower parts of the gut.

[0078] To a regular tube feeding is added after pasteurization a proportional amount of an aqueous pasteurized suspensión of encapsulated probiotics to obtain a final concentration of about 10^8 viable cells per ml.

Claims

1. Nutritional preparation with health promoting action, in particular with respect to the prevention and treatment of disorders of the gastrointestinal tract, comprising 10^6-10^{14}, preferably 10^7-10^{13} viable cells, per gram of the total preparation, of each of the following micro-organisms:

   - Bididobacterium;
   - Entercoccus faecium; and
   - a Lactobacillus strain that produces predominantly dextro-rotary lactate.

2. Nutritional preparation according to claim 1, comprising

   - Bifodobacterium infantis, Bifodobacterium globosum or Bifodobacterium lactis;
   - Enterococcus faecium, preferably strain SF 68
   - Lactobacillus rhamnosus, preferably strain ATCC 7469.

3. Nutritional preparation according to claim 1 and/or 2, further comprising 10^6-10^{14}, preferably 10^7-10^{13} viable cells, per gram of the total preparation, of a Lactococcus strain, preferably of Lactococcus lactis; or of a Micrococcus strain, preferably M. luteus.

4. Nutritional preparation according to any of the preceding claims, further comprising a prebiotic compound, preferably chosen from fibres that produce butyrate/butyric acid or propionate/propionic acid upon fermentation, such as resistant starch, gums, soy polysaccharides and/or proteins.

5. Nutritional preparation according any of the preceding claims, comprising at least 0,5 g fibre and/or at least 0,5 g yeast extract per 100 g of the total preparation.

6. Nutritional preparation according to any of the preceding claims, further comprising one or more substances that inhibits bacterial adhesion to the epithelial wall of the gastrointestinal tract, preferably chosen from the group lectins, glycoproteins, mannans, glucans, chitosan and/or derivatives thereof, charged proteins and/or adhesion-inhibiting immunoglobulins, modified carbohydrates and modified chitin, carob flower, as well as extracts which are rich in condensed tannin and tannin-derivatives, such as cranberry extract.

7. Nutritional preparation according to any of the preceding claims, further comprising

   - antibodies, such as immunoglobulins, that act specifically against the pathogenic micro-organisms, more specifically against enterotoxigenic E.coli strains, rotavirusses, Clostridia, Salmonella and/or Campylobacter species, and preferably in amounts of at least 20 µg/100 g of the total preparation;
   - sialylated compounds, preferably in amounts of at least 2 mg/100 g of the total preparation; and/or
   - a bactericidal compound, such as lactoferrin, preferably in amounts of at least 2 mg/100 g of the total preparation.
   - short chain fatty acids, preferably in a suitably

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EUROPEAN SEARCH REPORT

Application Number
EP 97 20 2900

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
A	MCFARLAND ET AL: "PHARMACEUTICAL PROBIOTICS FOR THE TREATMENT OF ANAEROBIC AND OTHER INFECTIONS" ANAEROBE, vol. 3, April 1997 - June 1997, pages 73-78, XP002057885 * page 73 * * abstract * ---	1-14	A61K35/74 A23L1/03
A	PATENT ABSTRACTS OF JAPAN vol. 11, no. 371 (C-462), 3 December 1987 & JP 62 145026 A (ADVANCE KK), 29 June 1987, * abstract * ---	1-14	
A	PATENT ABSTRACTS OF JAPAN vol. 12, no. 455 (C-548), 29 November 1988 & JP 63 179829 A (ADVANCE CO LTD), 23 July 1988, * abstract * ---	1-14	
A	DATABASE BIOSIS BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US abstract 76:178962, MOSKALIK ET AL: "INTERACTION OF MICROORGANISMS ARTIFICIALLY INTRODUCED INTO THE DIGESTIVE TRACT OF GNOTOBIOTIC CHICKS" XP002057886 * abstract * & IZV AKAD NAUK MOLD SSR SER BIOL KHIM NAUK, vol. 5, 1975, pages 36-40. -----	1-14	TECHNICAL FIELDS SEARCHED (Int.Cl.6) A61K

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	5 March 1998	Sitch, W

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